

That which is claimed is:

1. A method for producing a library of expressible coding regions comprising the steps of:

5 (a) amplifying a plurality of coding regions using at least one coding region specific primer,

(b) inserting each coding region into an expression vector, and

(c) verifying the size and orientation of the inserted coding region.

2. The method according to claim 1 further comprising transforming cells

10 with the vector containing the verified coding region.

3. The method according to claim 1 further comprising purifying the amplified coding region prior to insertion into an expression vector.

4. The method according to claim 1 wherein the coding regions encode full-length proteins.

15 5. The method according to claim 4 wherein the 5' primer used for amplification starts with the nucleotides CACCATG and the 3' primer causes the amplification product to end at the third position of the codon immediately preceding the stop codon of the coding region being amplified plus a single adenine residue.

6. The method according to claim 3 wherein the purification is performed  
20 using agarose gel electrophoresis.

7. The method according to claim 6 wherein the agarose is low melt agarose.

8. The method according to claim 1 wherein insertion of the amplified coding region into an expression vector is performed using an enzyme that both cleaves and ligates DNA.

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9. The method according to claim 3 wherein the purification is performed using low melt agarose gel electrophoresis and insertion of the amplified coding region into an expression vector is performed using an enzyme that both cleaves and ligates DNA.

5 10. The method according to claim 8 wherein said enzyme is a type I topoisomerase or a site-specific recombinase.

11. The method according to claim 10 wherein said enzyme is vaccinia DNA topoisomerase, lambda integrase, FLP recombinase or P1-Cre protein.

12. A method according to claim 11 wherein said enzyme is vaccinia DNA  
10 topoisomerase.

13. The method of claim 1 wherein the expression vector is a eukaryotic expression vector.

14. The method of claim 13 wherein said eukaryotic expression vector is pYES2/GS or pcDNA3.1/GS.

15 15. The method of claim 1 wherein the expression vector is a prokaryotic expression vector.

16. The method of claim 15 wherein said prokaryotic expression vector is pBAD.

17. The method according to claim 1 wherein the expression vector comprises  
20 one or more elements selected from: a promoter-enhancer sequence, a selection marker sequence, an origin of replication, an affinity purification tag sequence, an inducible element sequence and an epitope-tag sequence.

18. The method of claim 1 wherein size and orientation of the insert is verified using a polymerase chain reaction protocol.

19. The method of claim 18 wherein said verification is performed using whole cell lysates.
20. The method of claim 1 wherein the coding regions to be amplified are open reading frame sequences in prokaryotic DNA or eukaryotic DNA.
- 5       21. The method according to claim 20 wherein the eukaryotic DNA is obtained from yeast or mammalian cells.
22. The method according to claim 1 wherein the coding regions being amplified encode members of a family of proteins.
- 10      23. The method according to claim 22 wherein the proteins are human proteins.
24. The method according to claim 23 wherein the family of proteins are kinases, phosphatases, transcription factors, oncogenes, or tumor suppressors.
25. The method according to claim 1 wherein steps (a) and (b) are performed in a multiwell microtiter plate.
- 15      26. The method according to claim 1 wherein coding regions of the correct size and in the correct orientation are robotically selected for transformation into cells for expression.
27. The method according to claim 2 comprising the additional step of verifying that the transformed cells express the coding region.
- 20      28. The method according to claim 2 wherein the transformed cells are eukaryotic cells or prokaryotic cells.
29. A method according to claim 28 wherein the eukaryotic cells are CHO cells or *S. cerevisiae* cells.

30. An expression library of coding regions produced according to the method of claim 1.

31. The library according to claim 30 wherein the coding regions encode yeast proteins.

5 32. The library according to claim 31 wherein the coding regions encode mammalian proteins.

33. The library according to claim 32 wherein the mammalian proteins are human proteins.

10 34. The library according to claim 33 wherein the human proteins are kinases, phosphatases, transcription factors, oncogenes, or tumor suppressors.

35. An expression library obtainable from the method of claim 1.

36. An expression vector pYES2/GS.

37. An expression vector pCDNA3.1/GS.

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38. A method for producing a library of expressible coding regions comprising the steps of:

- (a) amplifying a plurality of coding regions using PCR, wherein the 5' primer comprises the sequence CACCATG and the 3' primer causes the amplification product to end just prior to any stop codon,
- (b) purifying the amplified coding regions using low melt agarose electrophoresis,
- (c) inserting each of the purified coding regions into an expression vector using vaccinia DNA topoisomerase, wherein said expression vector comprises a promoter-enhancer sequence, a selection marker sequence, an origin of replication, an affinity purification sequence, and an epitope-tag sequence,
- (d) transforming bacterial cells with the insert containing expression vector,
- (e) growing the transformed cells and verifying the size and orientation of the inserted coding region,
- (f) selecting expression vectors containing inserted coding regions in the correct orientation for expression of the gene product, and
- (g) transforming cells for expression with said expression vectors.

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